Remarks

Applicants have herein canceled claim 2 without prejudice or disclaimer. Applicants reserve the right to pursue claims encompassing all canceled subject matter in subsequently filed continuing applications. Claim 28 has been amended to correspond to claims 21-27 by including recitation of "An isolated protein..." (instead of "A protein..."). No new matter has been added. Claims 21-28 are currently pending.

Claim Rejections - 35 USC § 101

Claims 21-28 were rejected under 35 U.S.C. § 101 allegedly because the claimed invention is "not supported by either a specific and substantial asserted utility or a well-established utility." *See*, Paper No.092004, page 3. In particular, it was stated:

> The instant specification puts forth that the polypeptide is useful in a screening method to determine what ligands may activate or inhibit the polypeptide and also to determine what the physiological effects of the polypeptide might be (see page 4). This proposed use lacks a specific and substantial utility. It is not a specific use because any integral membrane protein could be used in exactly the same way. Further, many polynucleotides are known in the art to encode polypeptides, yet the polypeptides have no known function or ligands. Any of these orphan clones could be used in the manner described in the specification for the claimed polynucleotide.

See, Paper No.092004, page 3.

Applicants respectfully disagree and traverse. First, Applicants respectfully disagree with the assertion that any integral membrane protein could be used in the manner described in the present application. For one, there are many types of integral membrane proteins which are quite distinct from the one described in the present specification. For example, receptor tyrosine kinases are integral membrane proteins which span the cell membrane once and, upon ligand binding and receptor dimerization, transduce signals through the cell membrane via activation of an intracellular kinase domain. *See, e.g.,* Voet *et al.*, "Biochemistry", page 1077 including Fig. 33-69, John Wiley & Sons, New York, NY (1990). As another example, channel proteins are integral membrane proteins which form aqueous pores in the cell membrane via multiple passes of the polypeptide through the cell membrane. Membrane proteins such as these usually function to shuttle ions in and out of the cell. *See, e.g.,* Alberts *et al.*, "Molecular Biology of the Cell", 2nd Ed., pages 312-314, Garland

Publishing, Inc., New York, NY (1989) (submitted herewith). As a final example, integrins, such as fibronectin receptor, are integral membrane proteins which span the cell membrane via heterodimeric polypeptide associations. Receptors such as these lack catalytic activity such as that found in receptor tyrosine kinases but, instead, serve important functions in cell-cell and cell-extracellular matrix adhesion. *See, e.g.,* Alberts *et al.*, pages 821-823 including Figure 14-52 (submitted herewith). In contrast, however, the G-protein coupled receptor described in the present application, as explained more fully below, is quite different from integral membrane proteins such as the above mentioned examples. As such, none of the other types of membrane proteins mentioned above "could be used in exactly the same way" as the protein of the present invention.

Moreover, Applicants respectfully emphasize that, in disclosure not cited by the Examiner, the specification does in fact provide the specific description that the Examiner asserts is lacking. Specifically, the very first page of the specification discloses in the first paragraph that the subject protein is a member of the G-protein coupled receptor family of proteins. Then, in the second paragraph, the specification gives an example of a specific type of G-protein coupled receptors. In particular, this paragraph states, "Some examples of these proteins include the GPC receptors, such as those for adrenergic agents... (Kobilka, B.K., et al., PNAS, 84:46-50 (1987); Kobilka, B.K., et al., Science, 238:650-656 (1987)..." These two Kobilka references cited in the passage quoted above are cited as references AQ and AR, respectively, in the Information Disclosure Statement (IDS) submitted on July 16, 2004. The description of adrenergic receptors is repeated on page 2, which states in the last paragraph, "Other examples of members of this family include... adrenergic... receptors..." Against this background of an explicit teaching of adrenergic receptors as a specific type of G-protein coupled receptors, the specification then discloses that the subject protein is a member of this class of adrenergic receptor proteins. In particular, Applicants respectfully direct the Examiner's attention to Figure 2. As stated in the brief description of this figure at page 6, third paragraph, "Figure 2 illustrates an amino acid alignment of the G-protein coupled receptor of the present invention and the human adrenergic α_2A receptor." Indeed, the specification states at page 2, fifth paragraph, "The protein exhibits the highest degree of homology to human α_2A adrenergic receptor with 25.387% identity and 51.084% similarity over a 331 amino acid stretch."

The pending Office Action also indicated that "[b]asic research, such as studying the properties of the claimed product or the mechanisms in which the product is involved, does not constitute a substantial utility." *See*, Paper No.092004, page 3. Although Applicants do not

agree with this standard, Applicants also submit that the present invention provides utility beyond "basic research". For example, as noted by the Examiner, "the specification puts forth that compounds that bind to and activate or inhibit the polypeptide of SEQ ID NO:2 are useful in the prevention and/or treatment of a variety of diseases including upper respiratory conditions, hypertension and mycardial diseases [*inter alia*] (see pages 4 and 5)." *See*, Paper No.092004, page 3. Moreover, the specification describes antibodies which bind the receptor as one type of compound providing utility as an antagonist; *i.e.*, to bind and inhibit the polypeptide of the invention.

Hence, Applicants disagree with the further statement in the present Office Action which asserts:

> A stated belief that a correlation exists between the polypeptides and any number of diseases is not sufficient guidance to use the claimed polynucleotides to treat and/or [diagnose] a particular disease; it merely defines a starting point for further research and investigation. The instant application has failed to provide guidance as to how one of skill in the art could use the claimed invention in a way that constitutes a specific or substantial utility. The proposed uses of the claimed invention are simply starting points for further research and investigation into potential practical uses of the claimed nucleic acids.

See, Paper No.092004, page 4, second and third paragraphs.

In this regard, Applicants note that the manner of making and using an invention disclosed in a specification must be accepted by the PTO "unless there is reason to doubt the objective truth of the statements contained therein." *In re Marzocchi*, 58 C.C.P.A. 1069, 439 F.2d 220, 223, 169 U.S.P.Q. 367, 369 (C.C.P.A. 1971), Revised Utility Guidelines, 64 FR at 71442, part II.B.4. In applying this rule to the utility requirement, the Federal Circuit has held:

> ...the PTO has the initial burden of challenging a presumptively correct assertion of utility in the disclosure. Only after the PTO *provides evidence showing that one of ordinary skill in the art would reasonably doubt the asserted utility* does the burden shift to the applicant to provide rebuttal evidence sufficient to convince such a person of the invention's asserted utility.

In re Brana, 51 F.3d 1560, 34 USQP2d 1436 (Fed. Cir. 1995) (emphasis added). In another case, the Federal Circuit set forth the standard for the PTO establishing a utility rejection:

> The PTO may establish a reason to doubt an invention's asserted utility when the written description "suggest[s] an inherently unbelievable undertaking involve[s] implausible scientific principles." *Brana*, 51 F.3d at 1566, 34 USPQ2D AT 1441, *SEE*

> *ALSO In re Eltgroth*, 419 F.2d 918, 164, USPQ 221 (CCPA 1970) (control of aging process).

In re Cortright, 49 U.S.P.Q.2d 1464, 1466 (Fed. Cir. 1999). In addition, the PTO's own guidelines provide:

> Any rejection based on lack of utility should include a detailed explanation why the claimed invention has no specific and substantial credible utility. Whenever possible, the examiner should provide documentary evidence (e.g., scientific or technical journals, excerpts from treatises or books, or U.S. or foreign patents) to support the factual basis for the prima facie showing of no specific and substantial credible utility. If documentary evidence is not available, the examiner should specifically explain the scientific basis for his or her factual conclusions.

Revised Utility Guidelines, 64 FR at 71442, Part II.B(2)(d)3.

In the present case, the pending Office Action has not made the required showing that even one, much less all, of the disclosed, specific and substantial utilities for the claimed polypeptides cited above would not be unbelieveable in light of the teachings of the specification. Indeed, the biological significance of the adrenergic receptor family has been well recognized in the art. For example, Applicants respectfully direct the Examiner's attention to Pepperl *et al.*, "Adrenergic Receptors" CRC Press (1994), included as reference AX in the Information Disclosure Statement submitted on July 16, 2004. Pepperl *et al.* clearly shows that the biological significance of the adrenergic receptors is well established. As stated in Pepperl *et al.* at page 45, paragraph 2, "In the most basic sense, the adrenergic receptors are the physiological sites of action of the catecholamines epinephrine and norepinephrine." Moreover, Pepperl states in the very first sentence, "The adrenergic receptors (ARs) comprise one of the largest and most extensively characterized families within the G-protein coupled receptor 'superfamily'". As another example, Applicants respectfully direct the Examiner's attention to Fraser *et al.*, 1989, J. Biol. Chem. 264: 11754-11761, included as reference AO in the IDS filed on July 16, 2004.

Furthermore, Applicants do not have to prove that a correlation exists between a particular activity and an asserted therapeutic use of a compound as a matter of statistical certainty or provide actual evidence of success in treating humans where such a utility is asserted. All that is required of Applicants is that there be a *reasonable* correlation between the biological activity and the asserted utility. *See Nelson v. Bowler*, 626 F.2d 853, 857 (C.C.P.A. 1980).

Given the detailed description of the structure and activity of the specified polypeptides and the disclosed homology to other members of the adrenergic receptor family, together with the

well recognized significance of the many members of this family already known in the art, one skilled in the art would have found the asserted utility for the protein of the subject invention credible upon reading the specification.

Claim 28 was also rejected on the basis that it "does not require that the polypeptide be isolated and therefore reads on a protein present in a human body..." *See*, Paper No.092004, page 4, last paragraph. Applicants have herein amended claim 28 to recite "An isolated protein..." Accordingly, the rejection of claim 28 has been obviated.

In view of the above explanations and amendment of claim 28, Applicants respectfully request that the rejection of claims 21-28 under 35 U.S.C. § 101 be reconsidered and withdrawn.

Claim Rejections - 35 USC § 112, first paragraph.

Claims 21-28 were rejected under 35 U.S.C. § 101 allegedly because the claimed invention is "not supported by either a specific and substantial asserted utility or a well-established utility for the reasons set forth above, one skilled in the art would not know how to use the claimed invention so that it would operate as intended without undue experimentation." *See*, Paper No.092004, page 5, second paragraph.

Applicants respectfully disagree and traverse. In particular, for the reasons provided herein (see above), Applicants submit that the claimed invention is, in fact, supported by a specific and substantial asserted utility or a well-established utility. Therefore, one skilled in the art would, in fact, know how to use the claimed invention so that it would operate as intended without undue experimentation.

Accordingly, it is respectfully requested that the rejection of claims 21-28 under 35 U.S.C. § 112, first paragraph, be reconsidered and withdrawn.

Conclusion

Entry of the above amendment is respectfully requested. The Examiner is invited to call the undersigned at the phone number provided below if any further action by Applicant would expedite the examination of this application.

If there are any fees not already provided for due in connection with the filing of this paper, please charge the fees to our Deposit Account No. 08-3425. If a fee not already provide

for is required for an extension of time under 37 C.F.R. § 1.136, such an extension is requested and the appropriate fee should also be charged to our Deposit Account.

Dated: Dec 22, 2004

Respectfully submitted,

By _____

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To:

Our parents, who encouraged us,
Our teachers, who enabled us, and
Our children, who put up with us.

Cover Art: One of a series of color studies of horse heart cytochrome *c* designed to show the influence of amino acid side chains on the protein's three-dimensional folding pattern. We have selected this study to symbolize the discipline of biochemistry: Both are beautiful but still in process and hence have numerous "rough edges." Drawing by Irving Geis in collaboration with Richard E. Dickerson.

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ates host cell transformation. v-*src* has therefore been termed an **oncogene** (Greek: *onkos*, mass or tumor).

What is the origin of v-*src* and what is its viral function? Hybridization studies by Michael Bishop and Harold Varmus in 1976 led to the remarkable discovery that *uninfected chicken cells contain a gene,* **c-*src*** ("c" for cellular), *that is homologous to* v-*src* (the two genes differ mainly in that c-*src* is interrupted by six introns, whereas v-*src* is uninterrupted). Moreover, c-*src* is highly conserved in a wide variety of eukaryotes that span the evolutionary scale from *Drosophila* to humans. This observation strongly suggests that c-*src*, which antibodies directed against $pp60^{v\text{-}src}$ indicate is expressed in normal cells, is an essential cellular gene. In fact, as we shall see below, *both* $pp60^{v\text{-}src}$ *and its normal cellular analog,* **$pp60^{c\text{-}src}$**, *function to stimulate cell proliferation.* Apparently, v-*src* was originally acquired from a cellular source by an initially nontransforming ancestor of RSV. By maintaining the host cell in a proliferative state (cells are usually not killed by RSV infection), $pp60^{v\text{-}src}$ presumably enhances the viral replication rate.

Viral Oncogene Products Mimic the Effects of Polypeptide Growth Factors and Hormones

In order to understand how oncogenes subvert the normal processes of cell division, we must first understand these processes. *Cell proliferation is stimulated by hormonelike polypeptide growth factors.* These **mitogens** (substances that induce mitosis), the best characterized of which are **epidermal growth factor (EGF)** and **platelet-derived growth factor (PDGF),** bind with high affinity to the extracellular domains of specific protein receptors that span the plasma membranes of certain types of cells (Fig. 33-69). In doing so, they activate the receptors' cytoplasmic domains to phosphorylate their target proteins which, in turn, are thought to act as intracellular messengers that stimulate cell division by as yet dimly perceived mechanisms. For example, **transforming growth factor α (TGF-α),** a protein synthesized by and required for the growth of epithelial cells, is produced in excessive amounts in the skin of individuals with **psoriasis,** a common skin disease characterized by epidermal hyperproliferation.

Many growth factor receptors are ***tyrosine-specific protein kinases; that is, they phosphorylate specific Tyr OH groups in their target proteins*** (Fig. 33-69). Most protein kinases, it should be noted, specifically phosphorylate Ser or Thr residues (recall, e.g., that phosphorylase kinase phosphorylates Ser 14 of glycogen phosphorylase; Section 17-3C); only about one phosphorylated amino acid residue in 2000 is Tyr. It is, nevertheless, becoming increasingly evident that tyrosine phosphorylation is of central importance in regulating a variety of basic cellular processes. Curiously, many activated tyrosine kinases phosphorylate themselves. This **autophosphorylation,** at least in several cases, further

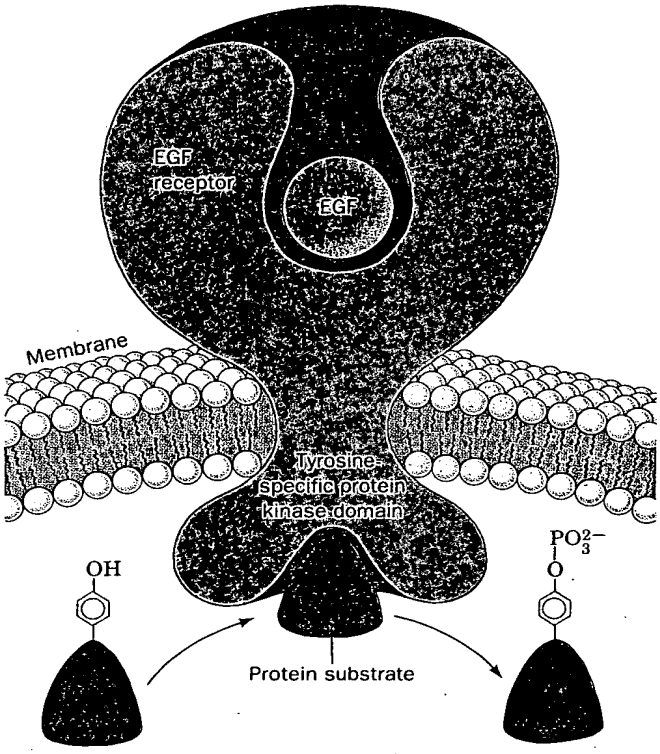

Figure 33-69
The binding of EGF to the external domain of EGF receptor activates its cytoplasmic tyrosine-specific protein kinase domain to phosphorylate specific Tyr residues of the receptor's protein substrates.

stimulates the tyrosine kinase activity of these activated receptors and therefore maintains the activated state after the growth factor has dissociated.

Hormones such as epinephrine and glucagon also profoundly affect the physiology of their target cells (Sections 17-3E–G and 34-4A and B). These hormones bind to specific receptors, thereby stimulating adenylate cyclase to catalyze the formation of cAMP, the second messenger that actually triggers the cellular response to the hormone. Hormone receptors, which face out from the plasma membrane, and adenylate cyclase, which is located on the membrane's cytoplasmic surface, are separate proteins that do not physically interact. Rather, they are functionally coupled by **G-protein** (Fig. 33-70), so-called because it specifically binds GTP and GDP. Adenylate cyclase is activated by G-protein but only when it is complexed with GTP. However, G-protein slowly hydrolyzes GTP to GDP + P_i and thereby deactivates itself. G-protein is reactivated by a GDP–GTP exchange reaction that is catalyzed by hormone–receptor complex but not by unoccupied receptor. *G-protein therefore mediates the hormonal signal.* This process is discussed in greater detail in Section 34-4B.

The proteins encoded by many viral oncogenes are ana-

MOLECULAR BIOLOGY OF
THE CELL

SECOND EDITION

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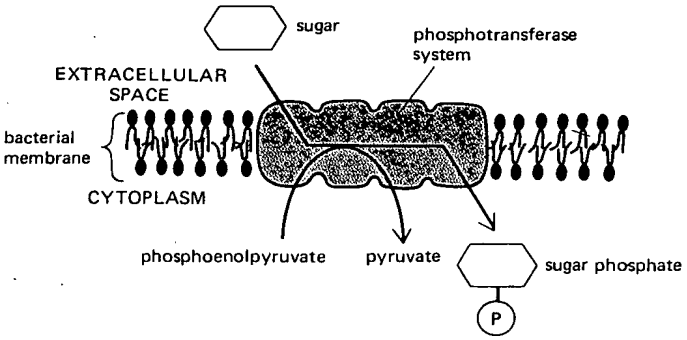

Figure 6–53 Active transport of sugars into bacteria by group translocation. A special "phosphotransferase system" of proteins in the bacterial membrane phosphorylates the sugar after the sugar's transport through the membrane. Phosphoenolpyruvate rather than ATP is the phosphate donor.

and highly regulated, involving at least four separate membrane proteins and phosphoenolpyruvate (rather than ATP) as the high-energy-phosphate donor (Figure 6–53).

Bacteria with Double Membranes Have Transport Systems That Depend on Water-soluble Substrate-binding Proteins[30]

As mentioned previously, the plasma membranes of all bacteria contain carrier proteins that use the H^+ gradient across the membrane to pump a variety of nutrients into the cell. But many bacteria, including *E. coli*, also have a surrounding *outer membrane*, through which solutes of up to 600 daltons can diffuse relatively freely through a variety of channel-forming proteins (known collectively as *porins*) (Figure 6–54). In these bacteria some sugars, amino acids, and small peptides are transported across the *inner (plasma) membrane* via a two-component transport system that utilizes water-soluble proteins located in the *periplasmic space* between the two membranes. These **periplasmic substrate-binding proteins** bind the specific molecule to be transported and, as a consequence, undergo a conformational change that enables them to bind to the second component in the transport system, which is a transmembrane carrier protein located in the inner membrane (Figure 6–55). It is thought that the substrate-binding proteins pass the bound solute to the carrier, which then uses the energy of ATP hydrolysis to transfer the solute across the inner membrane into the cell. The same periplasmic substrate-binding proteins serve as receptors in *chemotaxis*, an adaptive response that enables bacteria to swim toward an increasing concentration of a specific nutrient (see p. 720).

We now turn from carrier proteins to channel proteins.

Channel Proteins Form Aqueous Pores in the Plasma Membrane[31]

Unlike carrier proteins, **channel proteins** form water-filled pores across membranes. But whereas the channel-forming proteins of the outer membranes of bacteria (and of mitochondria and chloroplasts) have large, relatively unselective pores, channel proteins in the plasma membranes of animal and plant cells have

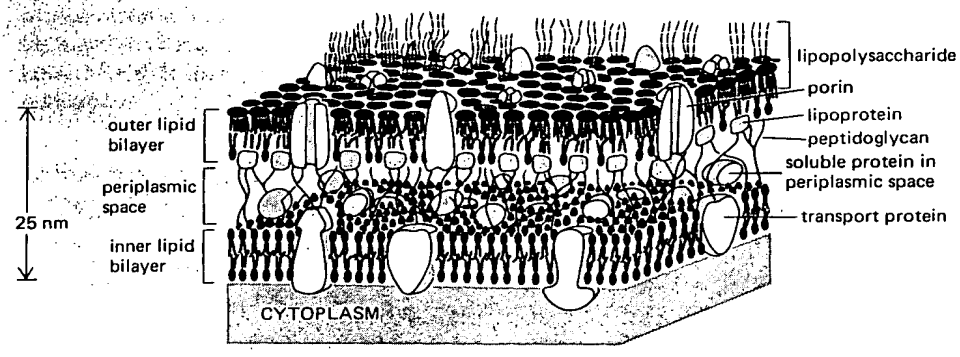

Figure 6–54 Schematic view of a small section of the double membrane of an *E. coli* bacterium. The inner membrane is the cell's plasma membrane. Between the inner and outer lipid bilayer membranes there is a highly porous, rigid peptidoglycan composed of protein and polysaccharide that constitutes the bacterial cell wall; it is attached to lipoprotein molecules in the outer membrane and fills the *periplasmic space*. This space also contains a variety of soluble protein molecules. The dashed black threads at the top represent the polysaccharide chains of the special lipopolysaccharide molecules that form the external monolayer of the outer membrane; for clarity, only a few of these chains are shown. Bacteria with double membranes are called *gram negative* because they do not retain the dark blue dye used in the gram staining procedure. Bacteria with single membranes (but thicker cell walls), such as staphylococci and streptococci, retain the blue dye and therefore are called *gram positive;* their single membrane is analogous to the inner (plasma) membrane of gram-negative bacteria.

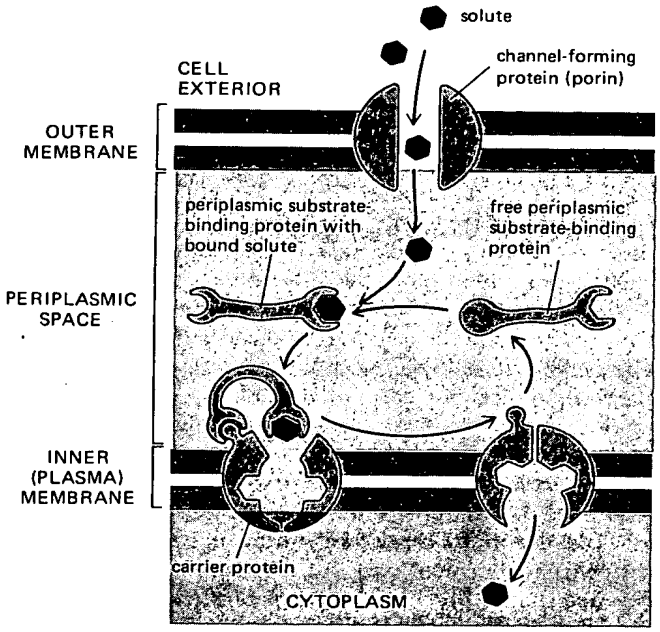

Figure 6–55 The transport system that depends on *periplasmic substrate-binding proteins* in bacteria with double membranes. The solute diffuses through channel-forming proteins (called *porins*) in the outer membrane and binds to a periplasmic substrate-binding protein. As a result, the substrate-binding protein undergoes a conformational change that enables it to bind to a carrier protein in the plasma membrane, which then picks up the solute and actively transfers it across the bilayer in a reaction driven by ATP hydrolysis. The peptidoglycan is omitted for simplicity; its porous structure allows the substrate-binding proteins and water-soluble solutes to move through it by simple diffusion.

small, highly selective pores. Almost all of the latter proteins are concerned specifically with ion transport and so are referred to as **ion channels.** More than 10^6 ions can pass through such a channel each second, which is a rate more than 100 times greater than the transport mediated by any known carrier protein. On the other hand, ion channels cannot be coupled to an energy source, so the transport they mediate is always passive ("downhill"), allowing specific ions, mainly Na^+, K^+, Ca^{2+}, or Cl^-, to diffuse down their electrochemical gradients across the lipid bilayer.

The channel proteins in plasma membranes show *ion selectivity,* permitting some ions to pass but not others. This suggests that their pores must be narrow enough in places to force permeating ions into intimate contact with the walls of the channel so that only ions of appropriate size and charge can pass. It is thought that the permeating ions have to shed most or all of their associated water molecules in order to get through the narrowest part of the channel. This both limits their maximum rate of passage and acts as a selective filter, letting only certain ions pass through. Thus, as ion concentrations are increased, the flux of ions through a channel increases proportionally but then levels off (saturates) at a certain maximum rate.

Another way in which ion channels differ from simple aqueous pores is that they are not continuously open. Instead they have "gates," which open briefly and then close again, as shown schematically in Figure 6–56. In most cases the gates open in response to a specific perturbation of the membrane. The main types of perturbations that are known to cause ion channels to open are a change in the voltage across the membrane (*voltage-gated channels*), mechanical stimulation

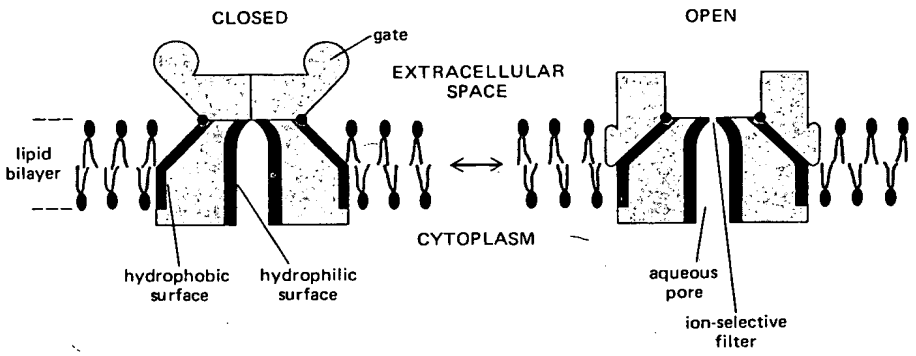

Figure 6–56 Schematic drawing of a gated ion channel in its closed and open conformations. A transmembrane protein, seen in cross-section, forms an aqueous pore across the lipid bilayer only when the gate is open. Hydrophilic amino acid side chains are thought to line the wall of the pore; hydrophobic side chains interact with the lipid bilayer. The pore narrows to atomic dimensions in one region (the "ion-selective filter"), where the ion selectivity of the channel is determined. A transient opening of the gate is caused by a specific perturbation of the membrane, which is different for different channels, as discussed in the text. The location of the gate and ion-selective filter, shown here on the external side of the membrane, is unknown for most channels.

(*mechanically gated channels*—see p. 1103), or the binding of a signaling molecule (*ligand-gated channels*). The signaling ligand can be either an extracellular mediator, called a *neurotransmitter* (*transmitter-gated channels*), or an intracellular mediator, such as an ion (*ion-gated channels*—see p. 1189), a nucleotide (*nucleotide-gated channels*—see p. 713), or a GTP-binding regulatory protein (*G-protein-gated channels*—see p. 706).

Approximately 50 types of ion channels have been described thus far, and new ones are still being discovered. They are responsible for the electrical excitability of nerve and muscle cells and mediate most forms of electrical signaling in the nervous system. A single nerve cell typically contains more than five kinds of ion channels. But these channels are not restricted to electrically excitable cells. They are present in all animal cells and are found in plant cells and microorganisms: they propagate the leaf-closing response of the mimosa plant, for example, and allow the single-celled paramecium to reverse direction after a collision.

Perhaps the most common ion channels are those that are permeable mainly to K^+ and are found in the plasma membrane of almost all animal cells. Because they seem not to require a specific membrane perturbation in order to open, they are sometimes called *K^+ leak channels*. Although poorly characterized and heterogeneous, they are the reason that most plasma membranes are much more permeable to K^+ than to other ions, and they play a critical part in maintaining the *membrane potential*—the voltage difference that is present across all plasma membranes.

6-24 6-26 The Membrane Potential Depends Largely on K^+ Leak Channels and the K^+ Gradient Across the Membrane

A **membrane potential** arises when there is a difference in the electric charge on the two sides of a membrane, due to a slight excess of positive ions over negative on one side and a slight deficit on the other. Such charge differences can result both from passive ion diffusion and from active electrogenic pumping. We shall see in Chapter 7 (p. 351) that most of the membrane potential of the mitochondrion is generated by electrogenic proton pumps in the mitochondrial inner membrane. Electrogenic pumps also generate most of the plasma membrane potential in plants and fungi. In typical animal cells, however, ion diffusion makes the largest contribution to the potential across the plasma membrane.

As explained earlier, the Na^+-K^+ ATPase helps to maintain osmotic balance across the animal cell membrane by keeping the intracellular concentration of Na^+ low. Because there is little Na^+ inside the cell, other cations have to be plentiful there to balance the charge carried by the cell's fixed anions—the negatively charged organic molecules that are confined inside the cell. This balancing role is performed largely by K^+, which is actively pumped into the cell by the Na^+-K^+ ATPase but can also move freely in or out through the **K^+ leak channels.** Thanks to the K^+ leak channels, K^+ comes very nearly to an equilibrium in which the electrical force due to negative charges attracting K^+ into the cell balances the tendency of K^+ to leak out down its concentration gradient. The membrane potential is the manifestation of this electrical force, and its size can be calculated from the steepness of the K^+ concentration gradient. The following argument may help to make this clear.

Suppose that initially there is no voltage gradient across the plasma membrane (the membrane potential is zero) but the concentration of K^+ is high inside the cell and low outside. K^+ will tend to leave the cell through the K^+ leak channels, driven by its concentration gradient. As K^+ moves out of the cell, it will leave behind negative charge, thereby creating an electrical field, or membrane potential, that will tend to oppose the further efflux of K^+. The net efflux of K^+ will halt if the membrane potential reaches a value where this electrical driving force on K^+ exactly balances the effect of its concentration gradient—that is, when the electrochemical gradient for K^+ is zero. Cl^- ions also equilibrate across the membrane, but because their charge is negative, the membrane potential keeps most of these diffusible ions out of the cell. This equilibrium condition, in which there is no net

At the site of neuromuscular contact (the synapse), the basal lamina has a chemically distinctive character, recognized, for example, by antibodies that bind to the lamina exclusively in this region. One of the functions of the junctional basal lamina, apparently, is to coordinate the spatial organization of the components on either side of the synapse. Evidence for the central role of the junctional basal lamina in reconstructing a synapse after nerve or muscle injury will be discussed in Chapter 19 (see p. 1124). These studies make it clear that we still have much to learn about the chemical and functional specializations of basal laminae. They also suggest that minor (but as yet undefined) components in the extracellular matrix may play a critical part in directing morphogenesis during embryonic development.

Integrins Help Bind Cells to the Extracellular Matrix[25]

To understand how the extracellular matrix interacts with cells, one has to define the cell-surface molecules that bind the matrix components as well as the extracellular matrix components themselves. As mentioned previously, some proteoglycans are integral components of the plasma membrane; their core protein may be either inserted across the lipid bilayer or covalently linked to it. By binding to most types of extracellular matrix components, these proteoglycans help link cells to the matrix. However, extracellular matrix components also bind to the cell surface via specific receptor glycoproteins. Because of the multiple interactions among matrix macromolecules in the extracellular space, it is largely a matter of semantics where the plasma membrane components end and the extracellular matrix begins. The glycocalyx of a cell, for example, often includes components of both (see p. 299).

The matrix receptors differ from cell-surface receptors for hormones and for other soluble signaling molecules in that they bind their ligand with relatively low affinity (K_a = 10^6–10^8 liters/mole) and are usually present at about 10- to 100-fold higher concentration on the cell surface. This suggests that the receptors might function cooperatively and that cells may respond to an organized group of ligands in the matrix rather than to individual molecules. In support of this suggestion, soluble cell-binding fragments of matrix components usually fail to elicit the cellular responses induced by the same components immobilized in a matrix.

A **fibronectin receptor** on mammalian fibroblasts is one of the best-characterized matrix receptors. It was initially identified as a plasma membrane glycoprotein that bound to a fibronectin affinity column and could be eluted with a small peptide containing the RGD cell-binding sequence (see p. 817). The receptor is a noncovalently associated complex of two distinct, high-molecular-weight polypeptide chains, called α and β. It functions as a transmembrane linker to mediate interactions between the actin cytoskeleton inside the cell and fibronectin in the extracellular matrix (Figure 14–52). We shall see later that these interactions across

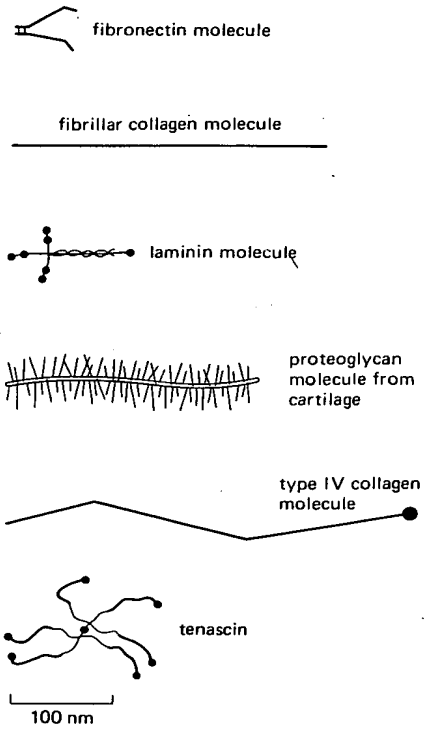

Figure 14–51 The comparative shapes and sizes of some of the major extracellular matrix macromolecules.

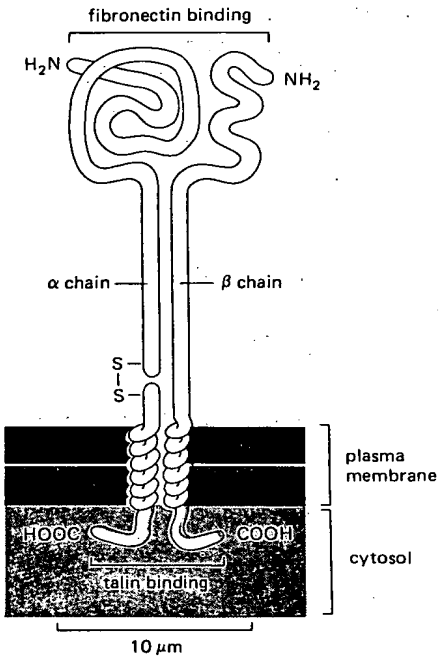

Figure 14–52 The subunit structure of a cell-surface fibronectin receptor. Electron micrographs of isolated receptors suggest that the molecule has approximately the shape shown, with the globular head projecting more than 20 nm from the lipid bilayer. By binding to fibronectin outside the cell and to the cytoskeleton (via the attachment protein talin) inside the cell, the protein serves as a transmembrane linker. The α and β chains are both glycosylated (not shown) and are held together by noncovalent bonds. The α chain is usually made initially as a single 140,000-dalton polypeptide chain, which is then cleaved into one small transmembrane chain and one large extracellular chain that remain held together by a disulfide bond. The extracellular part of the β chain contains a repeating cysteine-rich region, indicative of extensive intrachain disulfide bonding (not shown). The fibronectin receptor belongs to a large superfamily of homologous matrix receptors called *integrins*, most of which recognize RGD sequences in the extracellular proteins they bind.

the plasma membrane can orient both cells and matrix. Many other matrix receptors, including some that bind collagen and laminin, have been characterized and shown to be related to the fibroblast fibronectin receptor. Collectively called **integrins,** they are all heterodimers with α and β chains homologous to those of the fibronectin receptor. Most seem to recognize RGD sequences in the matrix components they bind.

There are at least three families within the large superfamily of integrins; the members of a family share a common β chain but differ in their α chains. One family includes a fibroblast fibronectin receptor and at least five other members. Another family includes a receptor found on blood platelets that binds several matrix components, including fibronectin and *fibrinogen,* which is a protein that interacts with platelets during blood clotting; humans with *Glanzmann's disease* are genetically deficient in these receptors and bleed excessively. A third family of integrins consists of receptors found mainly on the surface of white blood cells: one is called *LFA-1* (for lymphocyte function associated); another is called *Mac-1* because it is found mainly on macrophages. These receptors are involved in both cell-cell and cell-matrix interactions, and they are critically important in enabling these cells to fight infection. Humans with the disease called *leucocyte adhesion deficiency* are genetically unable to synthesize the β subunit. As a consequence, their white blood cells lack the entire family of receptors, and they suffer repeated bacterial infections. A number of cell-surface glycoproteins involved in position-specific cell adhesion in *Drosophila* larvae also belong to the integrin superfamily, but their relationship to the three families that are found in mammals is not certain.

Not all matrix receptors, however, belong to this superfamily. Some cells, for example, utilize an apparently unrelated transmembrane glycoprotein in binding to collagen; and many cells, as mentioned previously, have integral membrane proteoglycans that link cells to the extracellular matrix.

The Cytoskeleton and Extracellular Matrix Communicate Across the Plasma Membrane[26]

Extracellular matrix macromolecules have striking effects on the behavior of cells in culture, influencing not only their movement but also their shape, polarity, metabolism, and differentiation. Corneal epithelial cells, for example, make very little collagen when they are cultured on synthetic surfaces; but when they are cultured on laminin, collagen, or fibronectin, they accumulate and secrete large amounts of collagen. Other examples of extracellular matrix influences on cell metabolism and differentiation are discussed in Chapter 17 (see p. 987).

The matrix can also influence the organization of a cell's cytoskeleton. In general, the basal surfaces of epithelial cells cultured on plastic or glass are irregular, and the overlying cytoskeletons within the cells are disorganized. When the same cells are cultured on appropriate extracellular matrix macromolecules, the basal surfaces are smooth and the overlying cytoskeletons are highly organized, as they are in the intact tissue. Similar results have been obtained with neoplastically transformed fibroblasts in culture. Transformed cells often make less fibronectin than normal cultured cells and behave differently: for example, they adhere poorly to the substratum and fail to flatten out or develop the organized intracellular actin filament bundles known as *stress fibers* (see p. 627). In some of these cells, the fibronectin deficiency seems to be at least partly responsible for this abnormal behavior: if the cells are grown on a matrix of organized fibronectin fibrils, they will flatten and assemble intracellular stress fibers that are aligned with the extracellular fibronectin fibrils.

This interaction between the extracellular matrix and the cytoskeleton is reciprocal: intracellular actin filaments can influence the arrangement of secreted fibronectin molecules. In the neighborhood of cultured fibroblasts, for example, extracellular fibronectin fibrils assemble in alignment with adjacent intracellular stress fibers (Figure 14–53). If these cells are treated with the drug cytochalasin, which disrupts actin filaments, the fibronectin fibrils dissociate from the cell surface (just as they do during mitosis when a cell rounds up). Clearly there must be

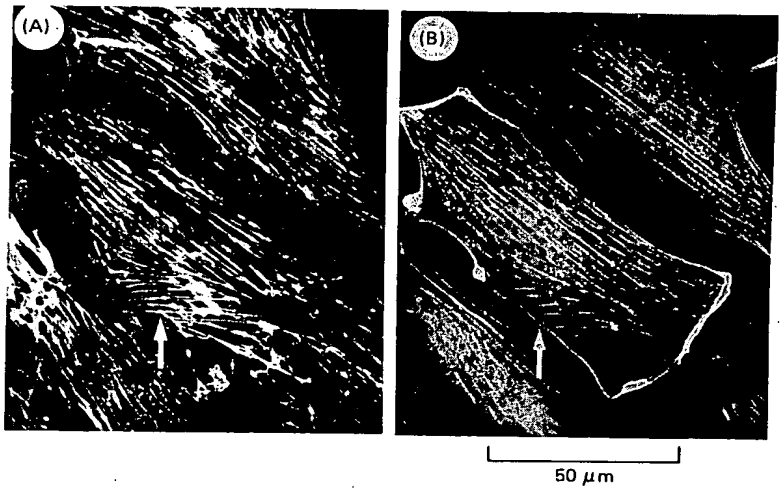

Figure 14–53 Immunofluorescence micrographs of extracellular fibronectin fibers (A) and intracellular actin filament bundles (B) in three rat fibroblasts in culture. The fibronectin is visualized by the binding of rhodamine-coupled anti-fibronectin antibodies and the actin by fluorescein-coupled anti-actin antibodies. Note that the orientation of the fibronectin fibers coincides with the orientation of the bundles of actin filaments. (From R.O. Hynes and A.T. Destree, *Cell* 15:875–886, 1978. © Cell Press.)

a connection between extracellular fibronectin and intracellular actin filaments across the fibroblast plasma membrane. The connection is mediated by the fibronectin receptors discussed previously, which serve as transmembrane linkers between fibronectin and intracellular actin filaments via a set of intracellular attachment proteins, including talin (see p. 636 and Figure 14–52). The part of the receptor that binds talin contains a tyrosine residue that, when phosphorylated by tyrosine-specific protein kinases, seems to inactivate the talin binding site, thereby breaking the link between fibronectin and cortical actin filaments. It is thought that the attachment of cells to the matrix may be regulated in this way by specific growth factors that activate tyrosine-specific kinases (see Figure 13–37, p. 758).

Since the cytoskeletons of cells can order the matrix macromolecules they secrete, and the matrix macromolecules can in turn organize the cytoskeletons of cells that contact them, the extracellular matrix can in principle propagate order from cell to cell (Figure 14–54). Thus the matrix is thought to play a central part in generating and maintaining the orientations of cells in tissues and organs during development: the parallel alignment of fibroblasts and collagen fibrils in tendons, for example, may in part reflect this type of interaction between cells and matrix. The transmembrane matrix receptors serve as "adaptors" in this ordering process, mediating the interactions between cells and the matrix around them.

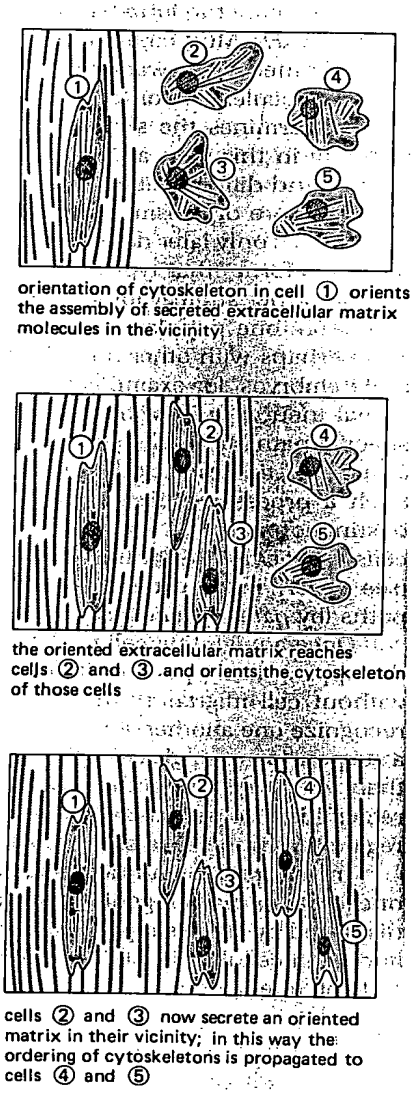

Figure 14–54 A hypothetical scheme showing how the extracellular matrix could propagate order from cell to cell within a tissue. For simplicity, the figure shows one cell influencing the orientation of its neighboring cells, but by this scheme cells could mutually affect one another's orientation.

Summary

Cells in connective tissues are embedded in an intricate extracellular matrix that not only binds cells and tissues together but also influences the development, polarity, and behavior of the cells it contacts. The matrix contains various fiber-forming proteins interwoven in a hydrated gel composed of a network of glycosaminoglycan chains. The glycosaminoglycans are a heterogeneous group of long, negatively charged polysaccharide chains, which (except for hyaluronic acid) are covalently linked to protein to form proteoglycan molecules.

The fiber-forming proteins are of two functional types: mainly structural (collagens and elastin) and mainly adhesive (such as fibronectin and laminin). The fibrillar collagens (types I, II, and III) are ropelike, triple-stranded helical molecules that aggregate into long cablelike fibrils in the extracellular space; these in turn can assemble into a variety of highly ordered arrays. Type IV collagen molecules assemble into a sheetlike meshwork that forms the core of all basal laminae. Elastin molecules form an extensive cross-linked network of fibers and sheets that can stretch and recoil, imparting elasticity to the matrix. Fibronectin and laminin are examples of large adhesive glycoproteins in the matrix; fibronectin is widely distributed in connective tissues, whereas laminin is found mainly in basal laminae. By means of their multiple binding domains, such proteins help cells adhere to and become organized by the extracellular matrix. Many of these adhesive glycoproteins